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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
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08/779,460 01/07/97 GODDIJN

0	U-011098-6
EXAMINER	

HM12/0330

WILLIAM R EVANS  
LADAS AND PARY  
26 WST 61ST STREET  
NEW YORK NY 10023

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DATE MAILED:

03/30/00

**Please find below and/or attached an Office communication concerning this application or proceeding.**

**Commissioner of Patents and Trademarks**

**Office Action Summary**Application No.  
**08/779,460**

Applicant(s)

**Goddijn et al.**

Examiner

**Ousama Zaghmout**

Group Art Unit

**1638**☒ Responsive to communication(s) filed on Oct 28, 1999☐ This action is **FINAL**.☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

**Disposition of Claims**☒ Claim(s) 24, 25, and 35-62 is/are pending in the application.Of the above, claim(s) 37, 38, 51-55, 57, and 58 is/are withdrawn from consideration.☐ Claim(s) \_\_\_\_\_ is/are allowed.☒ Claim(s) 24, 25, 35, 36, 39-50, 56, and 59-62 is/are rejected.☐ Claim(s) \_\_\_\_\_ is/are objected to.☐ Claims \_\_\_\_\_ are subject to restriction or election requirement.**Application Papers**☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.☐ The drawing(s) filed on \_\_\_\_\_ is/are objected to by the Examiner.☐ The proposed drawing correction, filed on \_\_\_\_\_ is ☐ approved ☐ disapproved.☐ The specification is objected to by the Examiner.☐ The oath or declaration is objected to by the Examiner.**Priority under 35 U.S.C. § 119**☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).☐ All ☐ Some\* ☐ None of the CERTIFIED copies of the priority documents have been  
☐ received.☐ received in Application No. (Series Code/Serial Number) \_\_\_\_\_.☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

\*Certified copies not received: \_\_\_\_\_

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).**Attachment(s)**☒ Notice of References Cited, PTO-892☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 11.5☐ Interview Summary, PTO-413☐ Notice of Draftsperson's Patent Drawing Review, PTO-948☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

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**DETAILED OFFICE ACTION**

1. The Group and/or Art Unit location of your application in the PTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1638.

2. The finality of the last office action mailed 2-1-1999 was withdrawn in view of the new restriction requirement presented by the Examiner during the personal interview of 28 October 1999 (See interview summary, Paper No. 19). The restriction requirement was as follows:

Restriction to one of the following inventions is required under 35 USC 121:

I. Claims 1,3-6, 9-12, 15-16, 24, 30-31 and 33-34, drawn to a method for producing trehalose comprising exposing plants transformed with a trehalose phosphate synthase gene to a chemical trehalase inhibitor, classified in class 800, subclass 284, for example.

II. Claims 8, 25-29 and 32, drawn to an antisense RNA-mediated method for inhibiting trehalase, classified in class 800, subclass 286, for example.

III. Claim 17, drawn to a plant gene encoding an enzyme involved in the trehalose biosynthetic pathway, classified in class 536, subclass 23.6, for example.

3. Applicants' response to the restriction requirement has been received (Paper No. 20).

Applicants have elected with traverse Group I. Under Group I, Applicants have listed newly

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added claims: 36, 39-42, 44-50, 56, 59-62 which correspond to claims of Group I as presented above under item number 1. The traversal is on the ground(s) that Applicants are entitled to a generic claim which reads on species of Groups I and II of the restriction requirement. In response to this argument, the restriction requirement has been converted to an election of species in view of the newly presented claims as follows:

This application contains claims directed to the following patentably distinct species of the claimed invention:

A. A method for producing trehalose comprising exposing plants transformed with a trehalose phosphate synthase gene to a chemical trehalase inhibitor (claims 36, 39-42, 49-50, 59-60);

B. An antisense RNA-mediated method for inhibiting trehalase (claims 37-38, 51-55, 57-58).

Applicant is required under 35 U.S.C. 121 to elect a single disclosed species for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, claims 24-25, 35, 43-48, 56, 61-62 are generic.

Applicant is advised that a reply to this requirement must include an identification of the species that is elected consonant with this requirement, and a listing of all claims readable thereon, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

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Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which are written in dependent form or otherwise include all the limitations of an allowed generic claim as provided by 37 CFR 1.141. If claims are added after the election, applicant must indicate which are readable upon the elected species. MPEP § 809.02(a).

Should applicant traverse on the ground that the species are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the species to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other invention.

The inventions of species A and B have different modes of operation, different functions, and different effects, as each of the species is drawn to a particular process with different cellular effects not required by the other, such as the claimed trehalase inhibitor and topical application of species A versus the gene encoding a trehalase-inhibiting product and transformation therewith of species B.

In order to expedite the prosecution, the claims corresponding to Group I which was elected by Applicants in Paper No. 20 were examined on the merits in this case.

**Claim Rejections - 35 USC § 112**

The following is a quotation of the first paragraph of 35 U.S.C. 112:

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The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

**1st paragraph**

Claims 24-25, 35-36, 39-50, 56, 59-62 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for inhibiting trehalose degradation by applying the trehalase inhibitor validamycin A to transgenic plants that express the trehalose phosphate synthase (TPS) gene from E.coli (pMOG799) (page 16 of the specification; page 21 Table 1, page 21), does not reasonably provide enablement for inhibiting trehalase activity in untransformed or transgenic plants by any trehalase inhibitor other than validamycin A in any plant species that is genetically altered to synthesize trehalose by the introduction of any gene from any source under any condition; and does not provide enablement for any plant species that naturally synthesize trehalose. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

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The specification only provides guidance for the obtention of trehalose-producing plants following transformation with an E.coli gene encoding trehalose phosphate synthase, followed by treatment of the transformed plants with trehalase inhibitor validamycin A. No guidance is presented regarding plants which naturally synthesize trehalase, and no guidance is presented for the identification and isolation of any other gene from any other source encoding an enzyme which would confer trehalase-synthesizing ability to a plant. In addition, no guidance is presented regarding the use of any trehalase inhibitor other than validamycin A. In contrast, the claims are broadly drawn to any naturally occurring plant which can produce trehalose, any type of genetic modification thereof, and any type of trehalase inhibitor.

The synthesis of trehalose in plants is unpredictable. The specification does not teach if the ability to synthesize trehalose is present in all plant species, if trehalose-synthesizing enzymes are the same in all plant species, if genes encoding trehalose-synthesizing enzymes are the same in all plant species, if synthesis of trehalose in plants takes place via a single step involving one single enzyme or multiple steps entailing the use of more than one enzyme encoded by more than one gene, if the genetically altered plants can be produced by transformation with a vector comprising a TPS gene, from a non-E.coli source or any other transgene; if the trehalase inhibitor would not be inactivating the transcriptional activity of the promoter which directs the expression of the gene encoding trehalose synthesizing enzyme. Furthermore, the specification does not teach a TPS gene from a source other than E.coli, or if the expression of such a gene would result in an increase in the amount of trehalose in plants. In addition, the specification does not teach if an

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optimization of the coding sequence is required for exogenous genes prior to their usage in making the genetic alteration as claimed in claim 35. In that respect, the specification does not disclose any step on the mutagenesis, modification, the alteration of the coding sequence around the translation initiation site to accommodate a Kozak consensus sequence. Moreover, modification of the coding sequence to enhance the expression of a non-plant gene in plants requires many steps which have not been addressed in the instant disclosure which include: changes in the localization of the regions of A+T richness to resemble the plant introns, and the optimization of the potential plant polyadenylation signal sequences, ATTTA sequences to avoid any destabilization of the mRNA in the plant.

In addition, the introduction of single genes which encode a single enzyme in a metabolic pathway may be insufficient to effect the desired phenotype in the transformed plants, due to feedback inhibition, improper levels of various substrates or precursors, or the influence of other factors on the phenotype. Smith et al. failed to observe the desired retardation of fruit softening in tomato plants transformed with antisense DNA to the polygalacturonase gene, presumably due to the involvement of other unknown factors (Smith et al. Nature. 1988. Vol. 334 (25): 724-726; see, e.g., page 725, paragraph bridging the columns). Furthermore, the process of modifying carbohydrate accumulation in transgenic plants is particularly unpredictable. See Kossmann et al (1995; Progress in Biotechnology, Volume 10), who teach the <sup>lack of</sup> influence of antisense potato starch accumulation genes on branching or phosphate content of starch (page 275, third through fifth full paragraph), the difficulty inherent in isolating individual starch synthesis enzymes or their

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corresponding genes (paragraph bridging pages 275 and 276), and the lack of correlation between reduction of branching enzyme gene activity and branching of starch in transgenic plants (see, e.g., page 277, penultimate paragraph).

Taken together, the instant disclosure lacks the proper and sufficient guidance to enable the claims as set forth. Subsequently, it is unpredictable if trehalose accumulation would have taken place in genetically altered plants other than those exemplified in the specification and if said plants would have shown an increased stress tolerance following treatment with any trehalase inhibitor.

In addition, the specification teaches that wild-type (untransformed) potato plants showed no trehalose accumulation with or without validamycin A (page 21, Tables 1 and 2, bottom row).

Thus, it appears that claims drawn to plants which naturally synthesize trehalose (claim 35, preamble; claims 46-47; and dependents) are not enabled. The claims are so broad that they

encompass any trehalase inhibitor. The Examiner would like to draw the attention of the Applicants to the fact that enzyme inhibitors fall into two broad classes: those causing irreversible inactivation of enzymes and those whose inhibitory effects can be reversed. Inhibitors of the first class usually cause an inactivating, covalent modification of enzyme structure. The kinetic effect of irreversible inhibitors is to decrease the concentration of active enzyme, thus decreasing the maximum possible concentration of ES complex. Since the limiting enzyme reaction rate is often  $k_2[ES]$ , it is clear that under these circumstances the reduction of enzyme concentration will lead to decreased reaction rates. Note that when enzymes in cells are only partially inhibited by irreversible inhibitors, the remaining unmodified enzyme molecules are not distinguishable from

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those in untreated cells; in particular, they have the same turnover number and the same  $K_m$ .

Turnover number, related to  $V_{max}$ , is defined as the maximum number of moles of substrate that can be converted to product per mole of catalytic site per second. As such, irreversible inhibitors are usually considered to be poisons and are generally unsuitable for practical purposes. In addition, the Examiner would like to bring to the attention of the Applicants that reversible inhibitors can be divided into two main categories--- competitive inhibitors and noncompetitive inhibitors---with a third category, uncompetitive inhibitors, rarely encountered. Inhibitor Type:

A. Competitive Inhibitor: specifically binds at the catalytic site, where it competes with substrate for binding in a dynamic equilibrium- like process. In a case like this,  $V_{max}$  is unchanged;  $K_m$ , as defined by  $[S]$  required for  $\frac{1}{2}$  maximal activity, is increased. B. Noncompetitive Inhibitor: the inhibitor binds E or ES complex other than at the catalytic site. Substrate binding unaltered, but ESI complex cannot form products. Inhibition cannot be reversed by substrate. In this case,  $K_m$  appears unaltered;  $V_{max}$  is decreased proportionately to inhibitor concentration. C.

Uncompetitive Inhibitor: the inhibitor binds only to ES complexes at locations other than the catalytic site. Substrate binding modifies enzyme structure, making inhibitor- binding site available. Inhibition cannot be reversed by substrate. In this case, apparent  $V_{max}$  decreased;  $K_m$ , as defined by  $[S]$  required for  $\frac{1}{2}$  maximal activity, is decreased. As Applicants broadly claim any trehalase inhibitor, they fail to recognize that each inhibitor has a different biochemical criteria and mode of action as described above. This means that it is unpredictable how each one of these inhibitors will react upon contact with the enzyme and what the inhibition rate if any will take

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place. Furthermore, Applicants failed to address problems associated with all of the reversible inhibitors which is that when the inhibitor concentration drops, enzyme activity is regenerated. Usually these inhibitors bind to enzymes by non-covalent forces and the inhibitor maintains a reversible equilibrium with the enzyme. Taken together, Applicants failed to provide any description and enablement whatsoever for these claimed inhibitors of trehalase. Thus it is not readily predictable that the trehalose accumulation specifically disclosed will work with other inhibitors or other plants. Applicants have provided no specific guidance as to how to select inhibitors which will give the desired effect or provided guidance with regard to the technique to be used in the modification in these plants.

Thus, taken together, the instant disclosure lacks the proper and sufficient guidance to enable the claims as set forth. Thus it is not readily predictable that the genetic modification specifically disclosed will work with other genes or other plants. Applicant has provided no specific guidance as to how to select genes which will give the desired effect or provided guidance with regard to selection of other plants and/or the technique to be used in the modification of these plants. One wishing to practice the invention is left to proceed through trial-and-error to see what will work and what will not.

Given the claim breadth, unpredictability, and the lack of guidance as discussed above, undue experimentation would have been required by one skilled in the art to obtain and evaluate plants which naturally synthesize trehalose; to identify and isolate a multitude of non-exemplified

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trehalose-synthesizing enzymes and their corresponding genes from a multitude of sources; evaluate the ability of said genes to cause trehalose production in a multitude of plants transformed therewith; to evaluate the ability of a multitude of non-exemplified types of genetic modification, such as mutation, to cause trehalose production in plants; or to evaluate the ability of a multitude of non-exemplified trehalase inhibitors at a multitude of concentrations for their ability to enhance trehalose production in native or genetically altered plants.

**2nd Paragraph**

Claims 24, 42, 56 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

1. Claim 24 is indefinite for depending on claim 1, a canceled claim.
2. Claim 42 recites the limitation “said product is a chemical trehalase inhibitor” in lines 1-2. There is insufficient antecedent basis for this limitation in the claim. It appears that the applicants’ intention was to make claim 42 dependent on claim 36 rather than claim 37. If so, then “said product” should also be deleted from claim 42. A correction is respectfully requested.
3. Claim 56 is indefinite for the improper use of Markush groupings. The claim preamble recites plants, but the body of the claim includes plant parts such as nuts, “leafs” (it should be leaves), roots and tubers. As such, the claim recites not equivalent Markush groupings since plant and parts are not equivalent. A separate claim drawn to plant parts should be submitted.

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**Claim Rejections - 35 USC § 102**

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless --

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

1. Claims 24, 35, 36, 41, 42 (since it was assumed to depend upon claim 36), 43-44, 47-48, 49, 50, 56 and 59-60 are rejected under 35 U.S.C. 102(b) as being clearly anticipated by Kendall et al. (Phytochemistry, 1990. Vol. 29: 2525-2528).

The claims are drawn to a process for producing trehalose in plant or plant parts therefrom that are naturally synthesizing trehalose or are genetically altered to synthesize trehalose by inhibiting trehalase activity.

The claimed inventions read on Kendall et al. as follows:

Kendall et al teach the use of validamycin A as a specific trehalase inhibitor when applied to a variety of plant species, wherein trehalose production occurs naturally in some plants (page 2525, abstract; first paragraph of "Introduction"). Kendall et al. teach the accumulation of trehalose in tissue treated with validamycin A at a concentration of 100 nM and 10 mM (Fig. 2, page 2526). Kendall et al. teach the use of a plant, or a plant part for extracting trehalose (Material and Methods section, page 2527). Kendall et al. teach a process of forced extraction from said plant

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or plant part (page 2527 under “quantitative analysis of trehalose breakdown” of Material and Method section). Trehalase activity is involved in fungal disease (i.e., “stress”) tolerance (see page 2527, column 1, bottom paragraph). All elements of the claims are found in the reference.

2. Claims 46-47 are rejected under 35 U.S.C. 102(a) as being clearly anticipated by Hoekema et al (WO 9501446; date of publication: 01-12-1995).

The claims are directed to a tuber or micro-tubers of Solanum tuberosum containing trehalose. Hoekema et al teach potato transformed with pMOG799, pMOG663 and pMOG664 which contains trehalose (line 663.1), expressing TPS gene (See pages 21-26, Examples I, II and V). The stress resistance of claim 47 would have been an inherent property (see page 5, lines 30-32).

#### **Claim Rejections - 35 USC § 103**

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter *as a whole* would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 24-25, 35, 36, 39-50, 56, 59-62 are rejected under 35 U.S.C 103 (a) as being

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unpatentable over Hoekema et al (WO 9501446; date of publication: 01-12-1995) taken with Kendall et al. (Phytochemistry. 1990. Vol. 29: 2525-2528).

The claims are drawn to a process intended to enhance the accumulation of trehalose in tissues by treating a plant with the trehalase inhibitor. In addition, the claims are directed to plants that accumulate trehalose.

Hoekema et al teach trehalose production in potato transformed with pMOG799, pMOG663 and pMOG664 ( line 663.1), expressing TPS gene (See pages 21-26, Examples I, II and V). The reference teaches a detailed procedure for the transformation of potato, a method which can easily be used to transform other plant species ("Transformation of potato" section, pages 18-19). *Stress tolerance conferred by trehalose is also taught, as discussed above.*

Hoekema et al. do not disclose or expressly teach the use of validamycin A to inhibit trehalase and cause the accumulation of trehalose in a plant.

Kendall et al. teach the use of various concentrations of validamycin A (  $10^{-11}$  -  $10^{-5}$  M) in treating plant tissues to inhibit trehalase and cause the accumulation of trehalose (Figure 1, page 2526).

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Hoekema et al and Kendall et al., are combinable because they are from a similar problem solving area, viz., production of plants or plant parts which accumulate trehalose wherein said plants are resistant to stress. At the time of the invention, it would have obvious to a person of ordinary skill in the art to use the trehalose-producing plants taught by Hoekema et al, and modify them by treating with validamycin A as taught by Kendall in order to inhibit trehalase activity. The motivation for doing so would have been to maintain high level of trehalose in a plant as suggested by <sup>Hoekema</sup> et al. The disaccharide trehalose is a stress-protective and preservative agent in plants.

Therefore, it would have been obvious to combine Hoekema et al and Kendall et al to obtain the plants and the process of increasing trehalose production in plant as specified in claims 24-25, 35, 36, 39 -50, 56, 59-62. Thus the claimed invention would have been prima facie obvious as a whole to one of ordinary skill in the art at the time the invention was made, especially in absence of evidence to the contrary. The evidence of unexpected results (trehalose accumulation) by Applicants were limited to a single trehalase inhibitor, validamycin A; a single plant, potato; a whole plant; and presence of transgene encoding TPS from E.coli (pMOG799) (page 16 of the specification; page 21, table 1). Only transformed plants and validamycin A treatment resulted in trehalose production. However, wild-type (untransformed) potato plants showed no trehalose with and without validamycin (bottom row). See In re Lindner, 173 USPQ 356 (CCPA 1972) and In re Grasselli, 218 USPQ 769 (Fed. Cir. 1983) which teach that the evidence of nonobviousness should be commensurate with the scope of the claims.

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**Prima Facie-Obviousness**

It is the Examiner's position that all elements of the applicant's invention with respect to the claims are instantly disclosed or fully envisioned by the teaching of the references cited above.

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**Future Correspondence**

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Ousama M-Faiz Zaghmout whose telephone number is (703) 308-9438. The Examiner can normally be reached Monday through Friday from 7:30 am to 5:00 pm (EST).

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, L. Smith, can be reached on (703) 308-3909. The fax phone number for the group is (703) 305-3014.

Any inquiry of a general nature or relating to the status of this application should be directed to THE MATRIX CUSTOMER SERVICE CENTER whose telephone number is (703) 308-0196.

Ousama M-Faiz Zaghmout Ph.D  
March 17, 2000

DAVID T. FOX  
PRIMARY EXAMINER  
GROUP ~~180~~ 1638

*Received 7-22*  
*Acting SPE*